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Seed Train Process Intensification Strategy Offers Potential for Rapid, Cost-Effective Scale-Up of Biosimilars Manufacturing

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Abstract

A perfusion approach at N-1, where cells stay in the exponential growth phase throughout the entire culture duration, is becoming more common as a strategy for process intensification. This is because the higher cell densities it generates allows manufacturers to skip seed stages and reduce process transfer time through multiple bioreactor sizes, thus providing more cost-effective biologics production in smaller facilities. However, this N-1 perfusion approach requires optimization. In this article, we describe the development and proof-of-concept studies with single-use rocking motion perfusion bioreactors in which we have achieved a ten-fold increase in viable cell count in N-1 seed stage, compared to the fed-batch control process, in just 6–8 days. We also mention in detail how we inoculated a 50 L bioreactor production run using this intensified seed train and show comparable growth kinetics and yield with a control process, also at 50 L scale. Using this intensification approach in the future will help our manufacturing facility, the Biopharma Division of Intas Pharmaceuticals Ltd., reach 4000 L production-scale volumes with fewer process transfer steps, and without changing the feeding strategy or production bioreactors of our biologics' portfolio.

Introduction

The current costs and global accessibility of biologics and vaccines requires the biopharmaceutical industry to pursue rapid, yet economic development strategies. One approach for shortening manufacturing timelines and reducing the cost of goods is "process intensification." This strategy was originally pioneered in the United Kingdom by the process technology group at Imperial Chemical Industries (ICI) to reduce their plant size, capital investments, and overhead costs while increasing productivity.[1]

Today, upstream and downstream bioprocess intensification can be achieved in smaller facilities with fewer scale-up steps to rapidly produce large numbers of therapeutic doses. An intensified cell culture process could improve overall manufacturing yields to enable the use of smaller-volume bioreactors. For example, by increasing the final cell titer by 1 log, a potential scale-up could be reduced from 20,000 L to 2000 L. This would significantly impact facility footprints, production timelines, media, buffer, reagent, and plant utility costs, as well as allowing the transition from large stainless steel vessels to smaller, more cost-efficient single-use (SU) bioreactors.

One area where upstream bioprocessing can be intensified is in the seed train. This article discusses how SU rocking motion (RM) and stirred tank bioreactor technology is being integrated and tested at leading biosimilars product manufacturer, Intas Biopharmaceuticals Ltd., Biopharma Division (Intas), to develop an intensified cell culture seed train protocol.

Traditionally, the seed train for production of a biosimilar at Intas has five transfer stages (**Figure 1**) beginning with using shake flasks in the post-thaw expansion (N-4) and then moving through a 5 L rocking motion bioreactor (N-3) to seed 50 L and 500 L stirred tank bioreactors (N-2 and N-1) and ending in a production culture with a final volume of 4000 L (N). The perfusion approach at N-1 is being adopted by several major biopharma companies, including Bristol-Myers Squibb[2-4] and Roche[5], where cells stay in the exponential growth phase throughout the entire culture. Intas has performed a series of studies with SU RM perfusion bioreactors (working volume 25 L). The goal was to achieve at least 50×10^6/mL viable cell concentration (VCC) and >95% cell viability for seeding the production-scale main bioreactor (4000 L) at a higher initial VCC.

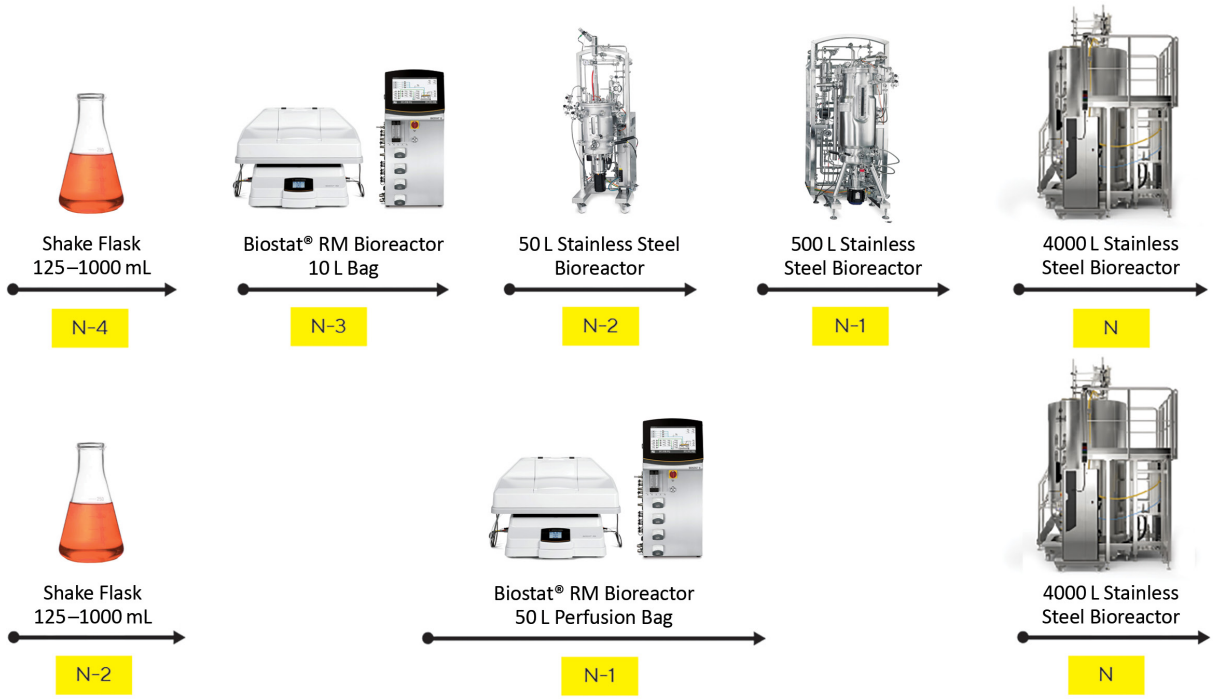

FIGURE 1. Traditional fed-batch (top) and perfusion (below) seed train cell culture process workflow.

Materials and Methods

Cell Line

A CHO-S cell line expressing a therapeutic monoclonal antibody (mAb), which is used as a commercial biosimilar, was chosen for the study. This cell line, its associated (proprietary) media, and culture parameters were selected for proof-of-concept experiments because the process has been well-characterized by Intas. Using this clone and media in an established 6–7 day fed-batch culture process that produces a 50–70 $\times 10^6$/mL VCC served as the benchmark which Intas scientists aimed for when adding perfusion culture into the seed train.

Bioreactors

The Biostat® RM 20/50 (Sartorius) was selected for the seed train studies because it is a fully GMP-compliant, rocking motion system where the surface of the medium is continuously renewed. This allows mass transfer between the headspace and medium for efficient mixing with low shear. SU Flexsafe® RM and stirred-tank (STR) bags (Sartorius) were used throughout the entire study. They are constructed of pharmaceutical-grade, low-density polyethylene with proven cell growth properties as the contact layer.[6] The Flexsafe RM perfusion bag features an integrated membrane at the bottom for cell-free removal of media during the culture process, with minimal cell loss or damage. Studies have shown that RM perfusion bioreactors can produce very high cell densities (≥40–100 $\times 10^6$/mL) at scales up to 25 L working volume.[7,8] This makes it possible to produce cells with high enough densities and viabilities to directly seed a production bioreactor. Additionally, the Biostat family of RM bioreactors scale from 10–100 L working volume, making process transfer simpler.

A Biostat STR 50 L bioreactor with SU Flexsafe STR bags was used in the pilot scale studies. This bioreactor comes in scales from 50–2000 L. A 4000 L stainless steel system served as the production bioreactor. The SU bioreactors uses the same established bioreactor design principles as the 4000 L stainless steel bioreactor, and all the STR bags have similar geometries across scales[9], which simplifies process transfer and scale-up.[10]

Trial Batches

Five seed train trial batch studies were performed to determine the optimum parameters for process intensification. CHO-S cells were cultured in shake flasks (125–500 mL volume) and used to seed growth media (5 L) in 10 L SU bags on the RM 20/50 bioreactor using a range of conditions (**Table 1**), media volumes, and inoculation densities (**Table 2**). Cells were sampled manually for analysis via the bioreactor bag's Luer connection using a sterile SU syringe. When the VCC reached >2.0–2.5 $\times 10^6$/mL, perfusion culture was started by continuous feeding and harvesting from a feed and harvest vessel (**Table 3**) until the minimum target VCC of >50 $\times 10^6$/mL was reached. VCC, cell viability, metabolites, glucose, and lactate were measured daily (where practicable) and used as performance indicators.

Proof-of-Concept Development

Cells cultured in the RM bioreactor (N-1) from batches 3 and 4 were used to seed two 50 L pilot batches (runs 1 and 2) in separate SU STR bioreactors. They were operated using different stir speeds, feed, and media to define the best culture parameters and yields.

Confirmation Run

After the intensification process was optimized at the N-1 perfusion stage, an additional confirmation run (batch 5) was carried out in the RM bioreactor for inoculation into a 50 L STR bioreactor (run 3). The culture performance of cells in the 50 L STR were compared with cells cultured in a control-seeded 50 L STR batch (inoculated using cells from a standard fed-batch culture). This served as a proof-of-concept to show how cells cultured using perfusion for process intensification performed against the traditional fed-batch seed train expansion methods.

TABLE 1. CHO-S cell culture conditions.

Parameters	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Inoculum VCC	0.5×10^6/mL	0.5×10^6/mL	0.5×10^6/mL	0.5×10^6/mL	0.5×10^6/mL
Inoculum cell viability	99%	99%	99%	99%	99%
pH post-inoculation	7.29	7.14	7.22	7.25	7.20
pH setpoint	7.00	7.00	7.00	7.00	7.00
Temperature	37°C	37°C	37°C	37°C	37°C
Initial DO	40%	40%	40%	40%	40%
Initial rocker speed (RPM)	16	16	16	16	16
Initial rocker angle	6°	6°	6°	6°	6°
Initial aeration flow rate O_2 (LPM)	0.1	0.1	0.1	0.1	0.1
Final rocker speed (RPM)	22	22	23	23	23
Final rocker angle	10°	9°	9°	10°	10°
Final aeration flow rate O_2 (LPM)	0.2	0.3	0.4	0.7	0.5

NOTE: Dissolved oxygen (DO), revolutions per minute (RPM), and liters per minute (LPM)

TABLE 2. CHO-S cell culture media volumes and inoculation densities.

Parameters	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Initial media volume	3.7 L	3.5 L	3.62 L	3.7 L	3.6 L
Inoculum volume	216 mL	200 mL	271 mL	300 mL	350 mL
Bioreactor volume post-inoculation	4.0 L	3.7 L	3.9 L	4.0 L	4.0 L
VCC post-inoculation (at 0 h)	0.59×10^6/mL	0.45×10^6/mL	0.45×10^6/mL	0.49×10^6/mL	0.48×10^6/mL
Cell viability post-inoculation (0 h)	99.0%	99.5%	99.5%	99.5%	99.2%
Batch duration (days)	6	10	9	8	6
VCC at harvest	14.5×10^6/mL	50×10^6/mL	88.5×10^6/mL	150×10^6/mL	64.4×10^6/mL
Cell viability at harvest	98.0%	98.9%	97.8%	97.2%	98.9%
Target VCC	50×10^6/mL	50×10^6/mL	50×10^6/mL	50×10^6/mL	60×10^6/mL
VCC when perfusion started	6.9×10^6/mL	2.6×10^6/mL	2.8×10^6/mL	3.11×10^6/mL	2.3×10^6/mL
Cell viability when perfusion started	99.0%	98.8%	99.8%	99.8%	98.9%

TABLE 3. CHO-S cell culture feed tank and harvest tank volumes.

Parameters	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Total volume of feed	12.0 L	22.0 L	38.4 L	50.0 L	50.0 L
Total volume of harvest	4.0 L	3.7 L	3.64 L	3.6 L	3.6 L

Results

DEVELOPMENT STUDIES

Performance of Batch 1

During the initial 3 days of this culture run, the VCC and cell viability of the CHO-S cells decreased by 1 log (from 0.50–0.06 ×10⁶/mL) and the run was halted. The culture was removed, and the bag washed with sterile water. New media (3.5 L) was added (pH 7.05) and this run was restarted. It was hypothesized that poor culture performance was caused by the cell culture media being sensitive to pH (7.5–7.7), which led to precipitation and arrested cell growth. For the rest of this batch run, pH was maintained at 7.0 to determine if this would improve VCC and cell viability.

During the remaining part of this 6-day culture run, the VCC increased by >1 log, from 0.6–14.5 ×10⁶/mL, and maintained 98% cell viability (**Table 4**), while glucose and lactate (measured from day 3 forward) were maintained between 2.0–2.5 g/L.

Performance of Batch 2

During the 10-day culture run of batch 2, the CHO-S VCC increased by 2 logs, from 0.45–50.00 ×10⁶/mL, and cell viability was 98.9% at harvest (**Table 5**). Glucose (measured from day 3 forward) was maintained at 0.5–2.0 g/L, and lactate decreased from 1.3–0.4 g/L.

Performance of Batch 3

To determine reproducibility, cell culture batch 3 was run for 9 days using similar parameters to batch 2. Perfusion was not carried out on day 9 (the final day of the run), so no volume was exchanged. The VCC and CHO-S cell viability increased by 2 logs, from 0.45–50.00 ×10⁶/mL (**Table 6**) with cells at 98.9% viability at harvest. Glucose (measured from day 3) was maintained at 0.50–2.77 g/L, and lactate decreased from 1.3–0.64 g/L. On day 7, 500 mL of batch 3 (perfusion culture) was taken from the 5.25 L harvested volume to inoculate 38 L of proprietary media in a 50 L STR bioreactor. Production run 1 reached a peak VCC of 9.76 ×10⁶/mL and 99.2% viability on day 11. The batch was harvested on day 16 with a VCC of 8.60 ×10⁶/mL and 96.6% viability (**Table 7**).

TABLE 4. CHO-S cell perfusion batch 1: VCC and cell viability data from a 6-day run.

Day	Initial Media Volume	Viable Cell Count	Cell Viability	Volume Taken/ Harvested	Volume Added	Perfusion Rate*	Rocker Speed	Rocker Angle
0	4.00 L	0.60 × 10⁶/mL	99.5%	0 L	0 L	0	16 RPM	6°
1	3.98 L	1.10 × 10⁶/mL	99.0%	0 L	0 L	0	16 RPM	6°
2	3.97 L	2.60 × 10⁶/mL	99.0%	0 L	0 L	0	17 RPM	6°
3	3.96 L	6.90 × 10⁶/mL	99.0%	4 L	4 L	1	18 RPM	7°
4	4.00 L	11.00 × 10⁶/mL	99.0%	4 L	4 L	1	19 RPM	7°
5	4.00 L	15.00 × 10⁶/mL	99.0%	4 L	4 L	1	20 RPM	8°
6	4.00 L	14.50 × 10⁶/mL	98.0%	0 L	0 L	0	21 RPM	10°

*Bioreactor volumes per day

TABLE 5. CHO-S cell perfusion batch 2: VCC and cell viability data from a 10-day run.

Day	Initial Media Volume	Viable Cell Count	Cell Viability	Volume Taken/ Harvested	Volume Added	Perfusion Rate*	Rocker Speed	Rocker Angle
0	4.00 L	0.45 × 10⁶/mL	97.0%	0 L	0 L	0.0	17 RPM	6°
1	3.98 L	0.70 × 10⁶/mL	99.0%	0 L	0 L	0.0	17 RPM	6°
2	3.97 L	1.50 × 10⁶/mL	98.5%	0 L	0 L	0.0	17 RPM	6°
3	3.96 L	3.20 × 10⁶/mL	98.8%	2.00 L	2.00 L	0.5	18 RPM	7°
4	4.00 L	6.00 × 10⁶/mL	99.1%	4.00 L	4.00 L	1.0	19 RPM	7°
5	4.00 L	9.91 × 10⁶/mL	98.8%	4.00 L	4.00 L	1.0	20 RPM	8°
6	4.00 L	17.10 × 10⁶/mL	99.3%	6.00 L	6.00 L	1.5	21 RPM	8°
7	4.00 L	27.10 × 10⁶/mL	99.7%	6.00 L	6.00 L	1.5	22 RPM	9°
8	3.50 L	40.90 × 10⁶/mL	99.8%	5.25 L	5.25 L	1.5	23 RPM	9°
9	3.50 L	45.00 × 10⁶/mL	99.2%	7.00 L	7.00 L	2.0	23 RPM	9°
10	3.50 L	50.10 × 10⁶/mL	98.9%	0 L	0 L	0.0	23 RPM	9°

*Bioreactor volumes per day

TABLE 6. CHO-S cell perfusion batch 3: VCC and cell viability data from a 9-day run.

Day	Initial Media Volume	Viable Cell Count	Cell Viability	Volume Taken/ Harvested	Volume Added	Perfusion Rate*	Rocker Speed	Rocker Angle
0	3.90 L	0.56×10^6/mL	98.7%	0.00 L	0.00 L	0.0	16 RPM	6°
1	3.88 L	ND	ND	0.00 L	0.00 L	0.0	16 RPM	6°
2	3.86 L	2.32×10^6/mL	99.7%	0.00 L	0.00 L	0.0	16 RPM	6°
3	3.84 L	4.28×10^6/mL	99.8%	2.20 L	2.20 L	0.5	18 RPM	6°
4	3.82 L	6.42×10^6/mL	99.5%	3.80 L	3.80 L	1.0	19 RPM	7°
5	3.80 L	12.50×10^6/mL	99.2%	3.80 L	3.80 L	1.0	19 RPM	7°
6	3.78 L	25.30×10^6/mL	99.6%	5.67 L	5.67 L	1.5	20 RPM	8°
7	3.76 L	41.80×10^6/mL	99.7%	7.50 L	7.50 L	2.0	21 RPM	8°
8	3.16 L	67.50×10^6/mL	98.7%	7.00 L	7.00 L	2.0	23 RPM	9°
9	3.14 L	53.50×10^6/mL	92.2%	0.00 L	0.00 L	0.0	23 RPM	9°

NOTE: No data (ND) *Bioreactor volumes per day

TABLE 7. 16-day production run 1 data: CHO-S cells cultured in the STR 50 L bioreactor seeded with batch 3 intensified (50 L perfusion) material.

Day	Viable Cell Count	Cell Viability	Lactate	Residual Glucose	pCO_2	mAb Titer Achieved
0	0.36×10^6/mL	99.4%	0.00 g/L	5.30 g/L	ND	
1	0.64×10^6/mL	99.5%	0.25 g/L	5.10 g/L	18.7 mmHg	
2	0.98×10^6/mL	94.4%	0.45 g/L	4.87 g/L	24.5 mmHg	
3	2.08×10^6/mL	96.3%	0.77 g/L	4.34 g/L	33.3 mmHg	
4	3.60×10^6/mL	98.8%	1.27 g/L	3.70 g/L	32.9 mmHg	
5	4.85×10^6/mL	99.8%	1.05 g/L	2.81 g/L	57.2 mmHg	
6	6.20×10^6/mL	99.7%	1.12 g/L	2.14 g/L	31.6 mmHg	
7	7.48×10^6/mL	99.7%	1.02 g/L	1.54 g/L	37.4 mmHg	
8	8.59×10^6/mL	99.5%	0.75 g/L	1.26 g/L	43.2 mmHg	
9	9.32×10^6/mL	99.6%	0.56 g/L	1.19 g/L	47.9 mmHg	
10	9.30×10^6/mL	99.2%	0.45 g/L	1.02 g/L	44.2 mmHg	
11	9.76×10^6/mL	99.2%	0.23 g/L	1.10 g/L	48.2 mmHg	
12	9.22×10^6/mL	99.1%	0.22 g/L	1.02 g/L	51.2 mmHg	
13	9.10×10^6/mL	98.8%	0.25 g/L	1.08 g/L	45.4 mmHg	
14	8.80×10^6/mL	98.8%	0.34 g/L	1.01 g/L	39.7 mmHg	
15	8.90×10^6/mL	98.8%	0.41 g/L	1.18 g/L	38.4 mmHg	
16	8.60×10^6/mL	96.6%	0.56 g/L	1.16 g/L	37.4 mmHg	1.18 g/L

Performance of Batch 4

CHO-S cell culture batch 4 was run for a total of 8 days. All conditions were similar to batches 2 and 3, with the exception of the perfusion rates, which were increased from 2–3 bioreactor volumes per day. The cells maintained good viability at 99.2–99.8% and VCC increased by 3 logs, from 0.49–150 $\times10^6$/mL (**Table 8**), which was more than double that of batches 2 and 3 at harvest. On day 7, 500 mL of batch 4 material was taken from the 7 L harvested volume to inoculate 38 L of proprietary media in a 50 L STR bioreactor. Production run 2 VCC reached a peak of 7.70 $\times10^6$/mL with 99.20% viability on day 9. The batch was harvested on day 16 with a VCC of 6.29 $\times10^6$/mL and 91.40% viability (**Table 9**).

Development Study Summary

Results from development batches 1–4 showed that the optimum intensified conditions for culturing CHO-S cells expressing a mAb biosimilar were: (1) 1–3 volumes per day of proprietary media in a culture run of 6–7 days; (2) a rocker speed increase from 16–23 RPM; (3) a rocker angle of 6–10°; and (4) a pH of 6.9 to prevent loss of cell viability. These optimized strategies were used for batches 2 and 3, achieving a VCC of 50–67 $\times10^6$/mL with 98% viability. Batch 4, where the perfusion rate was increased to 3 bioreactor volumes on day 6, achieved the best results, with a VCC of 150 $\times10^6$/mL and viability of 99.3% (**Figure 2**).

TABLE 8. CHO-S cell perfusion batch 4: VCC and cell viability data from an 8-day run.

Day	Initial Media Volume	Viable Cell Count	Cell Viability	Volume Taken/ Harvested	Volume Added	Perfusion Rate*	Rocker Speed	Rocker Angle
0	4.00 L	0.49×10^6/mL	99.5 %	0.0 L	0.0 L	ND	16 RPM	6°
1	3.98 L	0.71×10^6/mL	99.2 %	0.0 L	0.0 L	ND	16 RPM	6°
2	3.97 L	1.55×10^6/mL	99.3 %	4.0 L	4.0 L	1.0	16 RPM	6°
3	3.96 L	3.11×10^6/mL	99.8 %	6.0 L	6.0 L	1.5	16 RPM	6°
4	4.00 L	6.32×10^6/mL	99.5 %	8.0 L	8.0 L	2.0	19 RPM	8°
5	4.00 L	13.50×10^6/mL	99.4 %	10.0 L	10.0 L	2.5	20 RPM	9°
6	4.00 L	30.67×10^6/mL	99.5 %	12.0 L	12.0 L	3.0	21 RPM	10°
7	4.00 L	68.25×10^6/mL	99.5 %	12.0 L	12.0 L	3.0	22 RPM	10°
8	3.50 L	150.00×10^6/mL	99.3 %	11.5 L	11.5 L	3.0	23 RPM	10°

*Bioreactor volumes per day

TABLE 9. 16-day production run 2 data: CHO-S cells cultured in STR 50 L bioreactor seeded with batch 4 intensified material.

Day	Viable Cell Count	Cell Viability	Lactate	Residual Glucose	pCO_2	mAb Titer Achieved
0	0.52×10^6/mL	98.1 %	0.05 g/L	5.36 g/L	19.6 mmHg	
1	0.72×10^6/mL	99.1 %	0.19 g/L	5.31 g/L	42.2 mmHg	
2	0.91×10^6/mL	98.9 %	0.50 g/L	4.55 g/L	ND	
3	2.17×10^6/mL	98.2 %	1.10 g/L	3.36 g/L	31.0 mmHg	
4	4.20×10^6/mL	99.8 %	1.47 g/L	2.65 g/L	22.2 mmHg	
5	5.33×10^6/mL	98.9 %	1.29 g/L	2.05 g/L	34.8 mmHg	
6	6.75×10^6/mL	99.1 %	1.10 g/L	1.39 g/L	22.1 mmHg	
7	7.55×10^6/mL	98.7 %	0.72 g/L	1.15 g/L	42.5 mmHg	
8	7.12×10^6/mL	98.8 %	0.50 g/L	1.03 g/L	37.8 mmHg	
9	7.70×10^6/mL	98.5 %	0.34 g/L	1.01 g/L	51.9 mmHg	
10	7.35×10^6/mL	98.0 %	0.21 g/L	0.88 g/L	39.5 mmHg	
11	7.21×10^6/mL	99.2 %	0.23 g/L	1.10 g/L	48.2 mmHg	
12	7.20×10^6/mL	97.0 %	0.20 g/L	0.95 g/L	62.7 mmHg	
13	7.02×10^6/mL	97.0 %	0.08 g/L	0.94 g/L	67.3 mmHg	
14	6.85×10^6/mL	94.9 %	0.12 g/L	0.88 g/L	29.8 mmHg	
15	6.54×10^6/mL	93.6 %	0.34 g/L	1.29 g/L	31.3 mmHg	
16	6.29×10^6/mL	91.4 %	ND	ND	ND	1.20 g/L

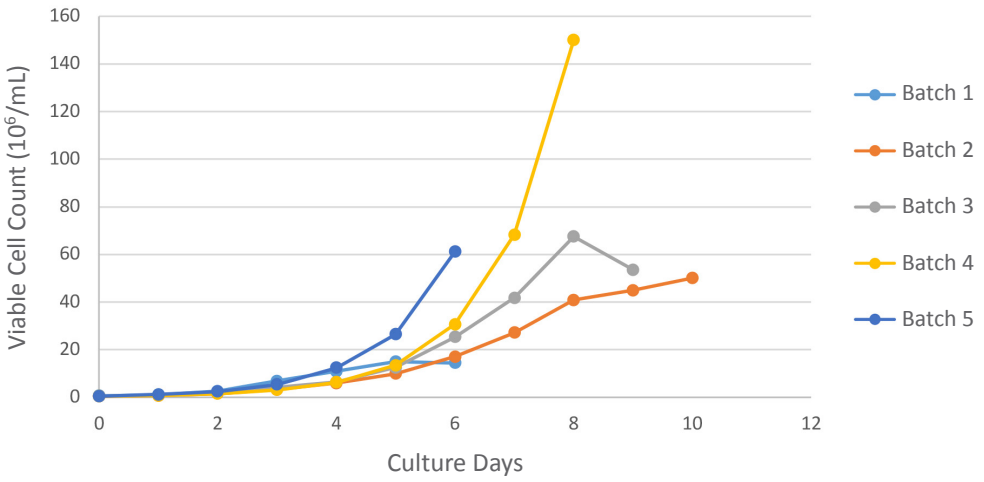

FIGURE 2. VCC of CHO-S cells in five perfusion culture development batch runs.

CONFIRMATION STUDY

Performance of Batch 5

Perfusion CHO-S cell culture batch 5 was run for 6 days with conditions similar to batch 4, which achieved the highest VCC, as compared to the previous four batches. The VCC for batch 5 reached 61.2 ×10⁶/mL while maintaining good viability, which was 99.3% on the final day 6 (**Table 10**). At that point, 500 mL of batch 5 was taken from the 9.5 L harvested volume to inoculate 38 L of proprietary media in a 50 L STR bioreactor. Production run 3 achieved a peak VCC of 9.30 ×10⁶/mL with a viability of 95.70% on day 13. The batch was harvested on day 16 with a VCC of 7.85 ×10⁶/mL and 97.20% viability (**Table 11** and **Figures 3A** and **3B**). These CHO-S cell culture results are comparable in performance (VCC, viability, glucose, and lactate) to batches produced by Intas following inoculation with the control cells expanded via fed-batch (**Table 11** and **Figures 4A** and **4B**). A sparge ventilation was used to remove headspace CO_2 in both runs, but pCO_2 levels in the control run oscillated more than the intensified run (**Figure 5**), as different proprietary flow rates were used to remove CO_2 in each run. Overall titers (**Figure 6**) and CQAs (data not shown) of the mAb product were also similar, with total galactosylation of 57% in the mAb derived from the control culture, compared to 54.5% from the intensified seed train culture.

Confirmation Study Summary

CHO-S cells cultured in the SU 50 L STR bioreactor performed well, following inoculation with the intensified (perfusion) seed train product, indicating that this approach can be used for a hybrid perfusion/fed-batch process scale-up.

TABLE 10. CHO-S cell perfusion batch 5: VCC and cell viability data from a 6-day run.

Day	Initial Media Volume	Viable Cell Count	Cell Viability	Volume Taken/ Harvested	Volume Added	Perfusion Rate*	Rocker Speed	Rocker Angle
0	4 L	0.48 × 10⁶/mL	99.0%	0.0 L	0.0 L	ND	16 RPM	6°
1	4 L	1.15 × 10⁶/mL	98.5%	0.0 L	0.0 L	ND	16 RPM	6°
2	4 L	2.30 × 10⁶/mL	98.8%	0.0 L	0.0 L	1.0	17 RPM	7°
3	4 L	5.35 × 10⁶/mL	99.4%	3.9 L	3.9 L	1.5	18 RPM	8°
4	4 L	12.40 × 10⁶/mL	99.3%	5.9 L	5.9 L	2.0	19 RPM	9°
5	4 L	26.50 × 10⁶/mL	99.4%	7.6 L	7.6 L	2.5	20 RPM	10°
6	4 L	61.20 × 10⁶/mL	99.3%	9.5 L	9.5 L	ND	20 RPM	10°

*Bioreactor volumes per day

TABLE 11. 16-day production run 3 data: A comparison of CHO-S cell culture performance using STR 50 L bioreactors seeded separately with the control (used as seed to inoculate the 4000 L fed-batch process) and intensified (50 L perfusion) materials.

Day	VCC (× 10⁶/mL)		Cell Viability (%)		Accumulated Lactate (g/L)		Residual Glucose (g/L)		pCO_2 (mmHg)		mAb Titer Achieved (g/L)	
	Control	50 L	Control	50 L	Control	50 L	Control	50 L	Control	50 L	Control	50 L
0	0.52	0.60	98.11	99.90	0.05	0.04	5.36	5.29	19.60	45.70		
1	0.72	1.20	99.10	99.40	0.19	0.19	5.31	5.25	42.20	87.10		
2	0.91	2.81	98.91	99.60	0.50	0.77	4.55	4.07	ND	34.40		
3	2.17	5.45	98.19	99.20	1.10	1.15	3.36	3.02	31.00	25.70		
4	4.20	6.88	99.76	99.80	1.47	1.17	2.65	2.97	22.20	32.40		
5	5.33	7.52	98.89	99.70	1.29	0.96	2.05	2.50	34.80	38.50		
6	6.75	7.55	99.12	99.60	1.10	0.74	1.39	2.43	22.10	52.90		
7	7.55	8.02	98.69	99.60	0.72	0.50	1.15	1.95	42.50	45.80		
8	7.12	9.12	98.80	99.20	0.50	0.32	1.03	1.66	37.80	52.70		
9	7.70	9.06	98.50	99.60	0.34	0.21	1.01	1.30	51.90	49.80		
10	7.35	8.72	98.00	99.60	0.21	0.10	0.88	1.37	39.50	54.20		
11	7.21	9.12	97.00	99.20	0.20	0.07	0.95	1.01	62.70	55.50		
12	7.20	8.51	97.00	99.20	0.08	0.07	0.94	1.27	67.30	52.10		
13	7.02	9.30	95.70	99.10	0.09	0.08	0.99	1.10	52.90	49.80		
14	6.85	8.19	94.90	99.00	0.12	0.09	0.88	1.20	29.80	51.20		
15	6.54	7.91	93.60	98.90	0.34	0.10	1.29	1.22	31.30	53.20		
16	6.29	7.85	91.40	97.20	ND	0.12	ND	1.20	ND	50.80	1.34	1.42

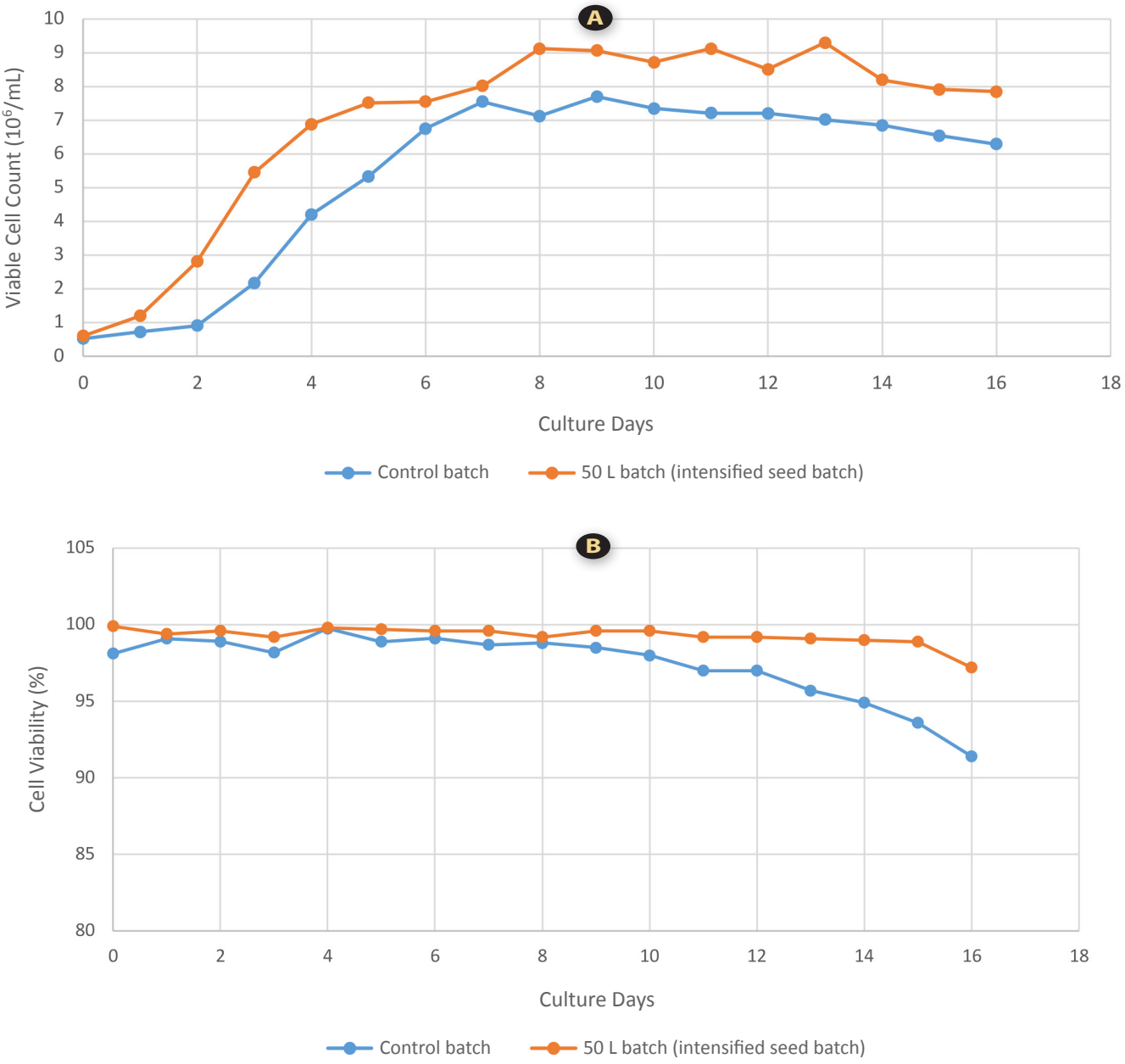

FIGURE 3. 16-day production run 3 data comparing: **(A)** VCC; and **(B)** cell viability in CHO-S cells cultured using STR 50 L bioreactors seeded separately with the control and intensified materials.

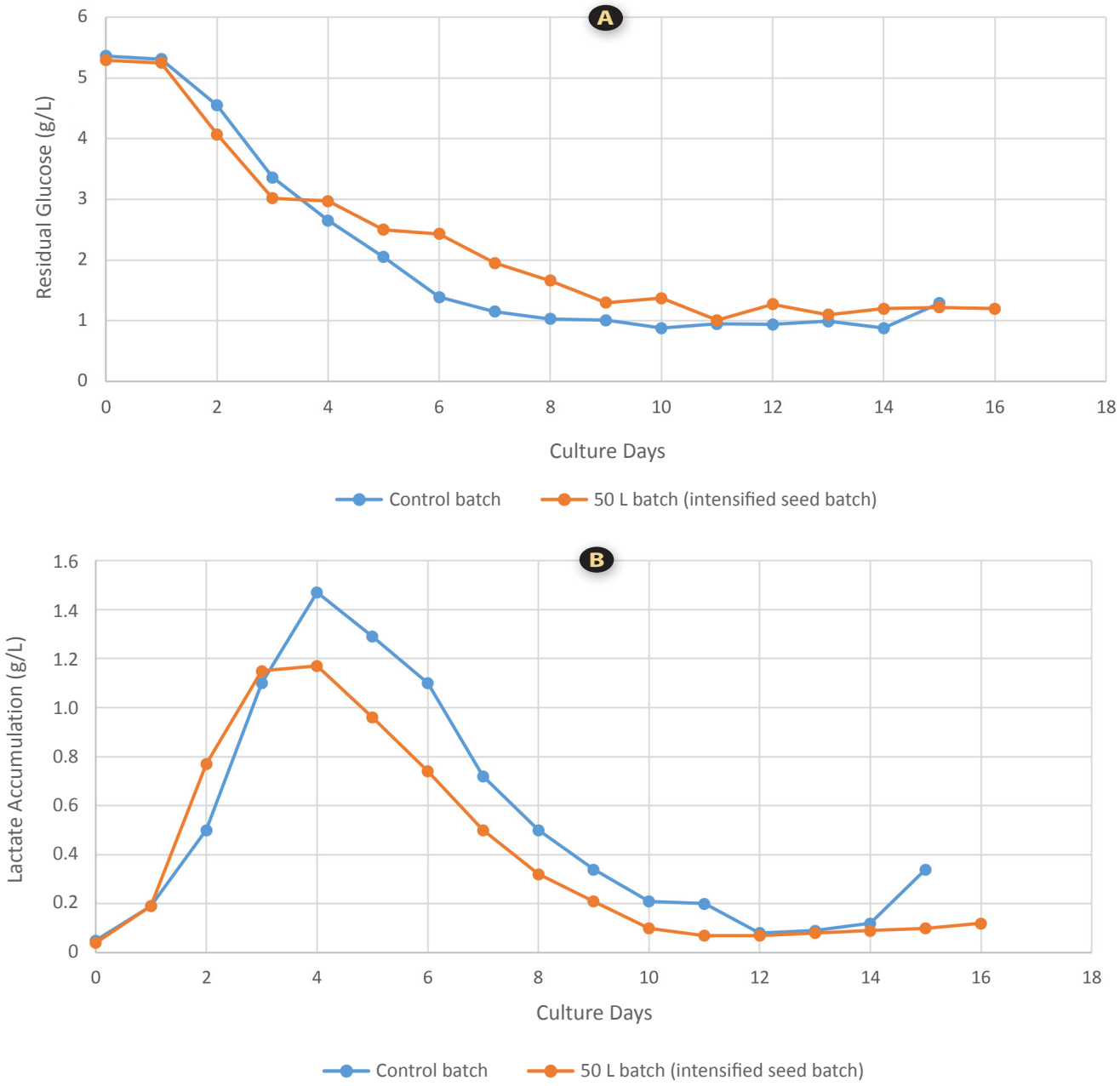

FIGURE 4. 16-day production run 3 data comparing: **(A)** residual glucose; and **(B)** lactate accumulation in CHO-S cells cultured using STR 50 L bioreactors seeded separately with the control and intensified materials.

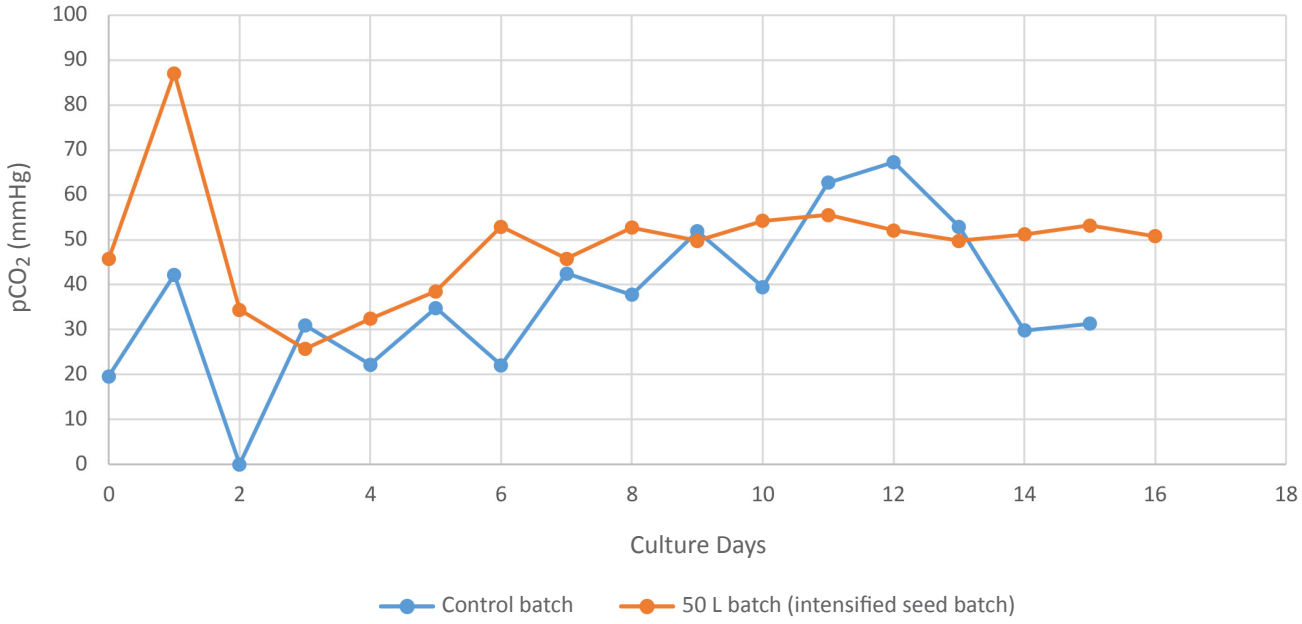

FIGURE 5. 16-day production run 3 data comparing pCO_2 in CHO-S cells cultured using STR 50 L bioreactors seeded separately with the control and intensified materials.

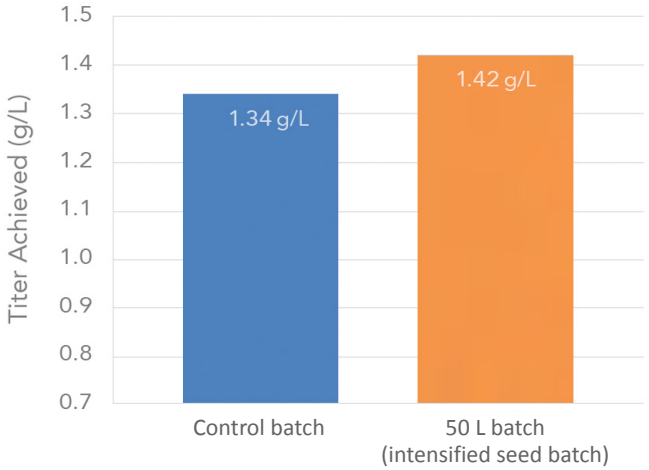

FIGURE 6. 16-day production run 3 data comparing CHO-S cell mAb titers using STR 50 L bioreactors seeded separately with the control and intensified materials.

Discussion

At Intas, when using a standard fed-batch seed train production protocol, the VCC typically reaches 7–8 ×10⁶/mL after 14–16 days of fed-batch culture with the CHO-S clone and media used as a control in this study. By intensifying the seed train cell expansion via perfusion technology, it is possible to achieve a ten-fold increase in VCC at N-1 seed stage compared to the control batch just described, in only 6–8 days. This is consistent with other studies where very high cell densities (≥40–100 ×10⁶/mL) scalable up to a working volume of 25 L are possible with CHO cells using perfusion culture intensification.[7, 8]

These results indicate that high cell densities can be achieved in less time using perfusion culture in the seed train. Production runs incorporating intensified seed trains also have comparable growth kinetics and yields, as compared to the control seed train process at 50 L scale. By using an RM 50 L perfusion bioreactor to generate cell batches at a VCC of 50 ×10⁶/mL, scientists at Intas could potentially seed a 4000 L bioreactor with 25 L of this material to achieve a target inoculation VCC of 0.4 ×10⁶/mL.

Conclusion

The development and proof-of-concept studies detailed in this article have shown that using SU RM perfusion bioreactors to produce seed train material could potentially help Intas achieve process intensification up to 4000 L with fewer process transfer steps. This could measurably increase productivity while consuming fewer resources. Other positives include reducing the manufacturing footprint without changing the production bioreactor or feeding strategy, potentially delivering the products in Intas' biologics portfolio more cost-effectively.

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